Influence of Microcystis sp. and freshwater algae on pH: changes in their growth associated with sediment.

C. Acuña, O. Lorenzo, X. Álvarez, Á. Cancela, E. Valero and Á. Sánchez

DOI: https://doi.org/10.1016/j.envpol.2020.114435

Influence of *Microcystis sp*. and freshwater algae on pH: changes in their growth associated with sediment.

Carolina Acuña[1], Olalla Lorenzo[2], Xana Álvarez[1], Ángeles Cancela[3], Enrique Valero[1] and Ángel Sánchez[4]

[1] Engineering Department of Natural Resources and Environment, School of Forestry Engineering, University of Vigo, Campus A Xunqueira s/n., 36005, Pontevedra, Spain. Tel.: +34-986-801959; e-mail: xaalvarez@uvigo.es (*X. Álvarez), evalero@uvigo.es (E. Valero), carolalonso12@gmail.com (C. Acuña).

[2]School of Forestry Engineering, Campus A Xunqueira s/n, University of Vigo, 36005, Pontevedra, Spain. E-mail: olallalorenzomiguez@gmail.com.

[3] Chemical Engineering Department, School of Forestry Engineering, University of Vigo, Campus CampusAXunqueira s/n., 36005, Pontevedra, Spain. e-mail: chiqui@uvigo.es.

[4] Chemical Engineering Department, Industrial Engineering College, University of Vigo, Campus Lagoas-Marcosende s/n, 36310, Vigo- Pontevedra, Spain; e-mail: asanchez@uvigo.es

ABSTRACT

Samples from two reservoirs with eutrophication problems, located in Pontevedra and Ourense (Northwestern Spain), were cultured, along with a third crop from a reservoir with no problems detected in Ourense (Northwestern Spain). The samples were grown under the same conditions (with an average temperature of 21±2°C, and a 3000 lux light intensity) in triplicate, and their growth, absorbance and pH were studied. High correlation values were obtained for pH and cellular growth ($R^2 \geq 95\%$). The water from Salas showed the greatest microalgal growth ($0.15x10^6$ cells/ml to $31.70x10^6$ cells/ml of *Microcystis sp*. for the last day of culturing) and the greatest increase in pH (5.72 to 9.02). In all the cultures studied here, the main species that reproduced was *Microcystis sp*., which can produce neurotoxins and hepatotoxins. In addition, water samples were cultured with sediments of their own reservoir and with others to observe their evolution. The sediments studied in this case were rich in biotites, which can lead phosphate to be a limiting factor for phytoplankton due to the formation and sedimentation of insoluble salts of ferric phosphate. In crops grown with sediments from the Salas reservoir, actinobacteria developed which can inhibit microalgal growth. The study of the growth of cyanobacteria and possible methods of inhibiting them directly concerns the quality of water and its ecosystems, avoiding pollution and impact on ecosystems.

KEYWORDS: Eutrophication, pH variations, microalgae, cyanobacteria, Water quality, *Microcystis aeruginosa.*

1. Introduction

Reservoirs are lentic ecosystems (Rodrigues, 2017) in which biodiversity is maintained via the functional balance between abiotic and biotic factors (Avery, 1996). A change in any of these factors can cause a change in the characteristics of this balanced habitat and in the populations of organisms that live in it (Marbello Pérez et al. 2012). One possible change is the deterioration of water quality because of eutrophication. This is a problem that affects water reserves worldwide, especially lakes and reservoirs (Dodds et al. 2008). Eutrophication is a natural process that can be accelerated by the external addition of a limiting abiotic factor such as nutrients in the form of fertilizers, especially nitrogen and phosphorus (Chislok et al. 2013). Differences in land use as a result of increasing levels of different anthropogenic pressures (Álvarez et al. 2017), runoff from wastewater to streams, destruction of riparian forest buffers (Palmer et al. 2000) and other possible causes may influence plant biomass growth. These factors are needed for photosynthesis (Schindler, 2006), but if they are present in excess the natural balance is broken and opportunistic species will benefit, e.g. primary producers such as microalgae, cyanobacteria and macrophytes. Consequently, blooms of these species cover the surfaces of lakes and reservoirs and modify aquatic ecosystems (Luna and Carmenate, 2009).

Research has shown that increases in CO_2 concentrations are associated with pH decrease, significantly influencing specific growth rates and the photosynthetic characteristics of freshwater microalgae, decreasing their growth (Yang and Gao, 2003) . However, the primary production rate exceeds the consumer grazing rate in episodes of excessive biomass growth, leading to decreased carbon dioxide concentrations in water. The removal of carbon dioxide from water increases pH as a result of reductions in carbonate and levels in water of bicarbonate, which is used to replace the lost carbon dioxide, due to the buffering capacity of water (Pérez and Restrepo, 2008).

At this point, the amount of carbon dioxide dissolved in water may not be enough to sustain the entire algal biomass and part of it will biodegrade. This will lead to harmful problems such as water quality deterioration, including foul odours and tastes, deoxygenation of bottom waters

(hypoxia and anoxia), toxicity, fish kills, and food web disturbances. Toxins produced by cyanobacteria blooms can adversely affect animal (including human) health in waters used for recreational and drinking purposes (Pearl et al. 2001).

The reservoirs of Galicia, in north-western Spain, are reported as areas with Cyanobacterial Harmful Algae Blooms (HABs) every year (Schmid et al. 2009). The reservoirs of As Conchas and A Baxe, studied in this paper, have HABs every year. Livestock and agriculture are important sectors in Galicia. In combination with its mountainous terrain and high rainfall, this may be why nutrients reach its rivers. These nutrients are accumulated in the reservoirs, favouring the proliferation of HABs and in consequence modifying the pH.

The main objective of this research is to analyse how increases in algal biomass modify pH in freshwater. To that end, microalgae blooms were simulated under laboratory conditions and algae growth from three different reservoirs was then compared and analysed.

After studying microalgae growth linked to pH, seeking to detect any particular features, we studied influence of sediment for two of the reservoirs considered: A Baxe and Salas. The objective was to find out why the Salas reservoir maintains a good ecological state despite all the extrinsic factors conducive to its eutrophication, once the reason was found to see if it was applicable to other eutrophicised waters to improve their state.

2. MATERIAL AND METHODS

2.1. Water samples and algae growth

The water samples used in the study were collected from algae concentrations on the surfaces of three reservoirs in line with standard UNE-EN16698:2016, on the 23rd and 24th of April 2017. The reservoirs are in Galicia (north-western Spain), as shown in Table nº1 and Figure 1.

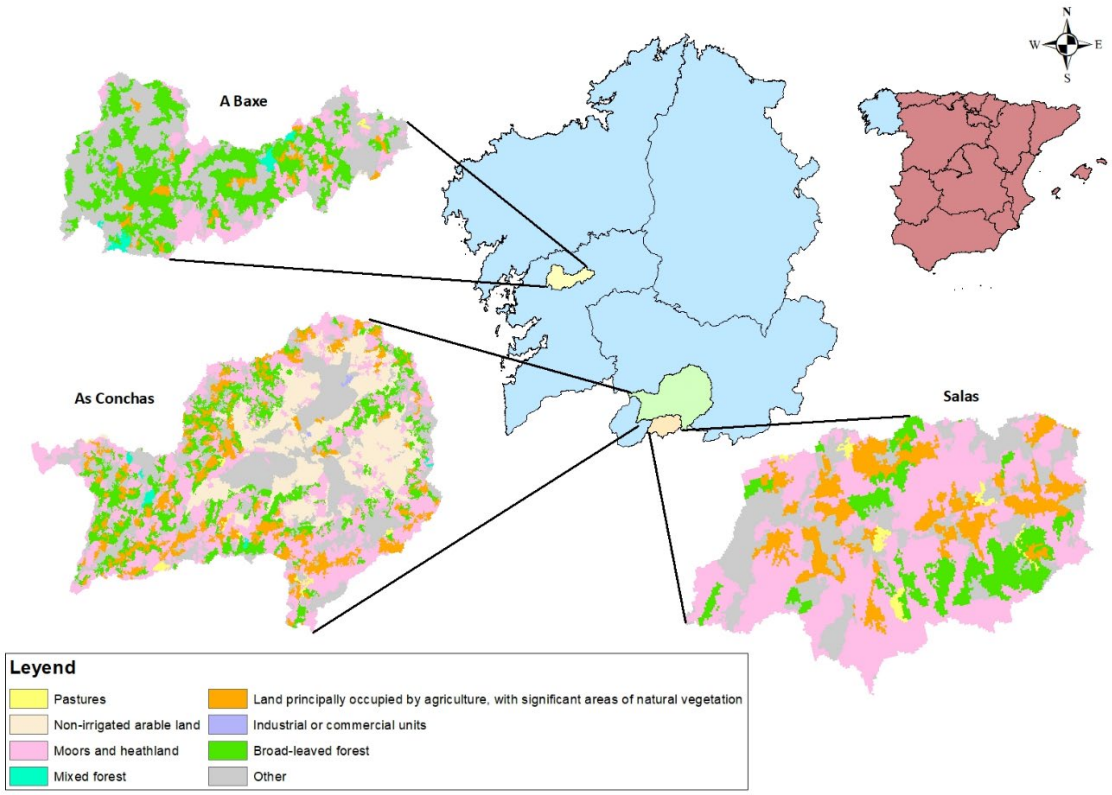


Figure 1: Geographic location of As Conchas, Salas and A Baxe basins. Drainage network and land uses (CORINE 2017) are also shown.

Three water samples of 600 ml each were cultured for each reservoir in a tubular methacrylate recipient with constant aeration by means of a mouse wave air pump (Mouse 4 aerator, Q=3,5 l/min, Wave). Microalgae were cultured under controlled conditions from April to June 2017 in a Guillard's culture medium (Guillard, 1975) at an average temperature of 21 ± 2 ºC for 25 days.

Cell growth was measured by means of the algal suspension absorbance at 690 nm, as described in Becker (1994). The absorbance values were measured every day at the same times in each culture with a SPECTRO 22 Digital Spectrophotometer.

The samples were identified and quantified as per UNE-EN 15204:2007. Taking into account that the water samples had *Mycrocystis sp*. colonies, a process of disaggregation was conducted as indicated in Janse et al. (2004). For this purpose, 20 ml of sample was collected, filtered with an HA membrane filter of 45 mm diameter and a pore size of 0.45 nm (Millipore, Bedford, MA).

The filter was transferred to an Erlenmeyer flask containing 20 ml of 0.01 M KOH and incubated for 30 minutes at 80 ° C.

Microalgae were identified microscopically and the cell count was obtained in a Neubauer chamber using optical microscopes (Kiowa Optical Medilux 12 and Binocular ZUZI 122/147 associated with a Moticam 5MP, Motic). Temperature (Checktemp 1 HI98509, Hanna) and pH (pH Meter basic 20, Crison) measurements were taken every day.

2.2. Algal growth based on sediment

The Salas reservoir features degraded vegetation, with livestock and agriculture adjacent to the reservoir and fires on the slopes every summer. Strikingly, its water maintains a good ecological state. In particular, we focused on the relationship with its surroundings, focusing on the sediment in the basin. The A Baxe reservoir has frequent cyanobacterial blooms which continually compromise the use of its water to supply the municipalities of Caldas de Reis (9.860 inhabitants in 2018) and Vilagarcía de Arousa (37519 inhabitants in 2018).

To analyse the influence of sediments on the proliferation of cyanobacteria and other algae, a series of crops were organized. First, a sample of water from the A Baxe reservoir was set up with sediments from the same reservoir and from the Salas reservoir. Then a culture with water form Salas was set up with sediments from the same location and sediments collected from the A Baxe reservoir. Finally, control cultures were set up with water samples from both reservoirs with no sediment.

To study the crops described above, the procedure described in subsection 2.1 was carried out, though in this case cell disintegration was not performed. In addition, the same tests already described were conducted: identification and quantification, pH and absorbance measurements.

The composition of the sediment of the two reservoirs and the ash collected at the foot of the Salas dam was analysed at the Laboratory of the Scientific Research Support Centre (CACTI) at the University of Vigo. The results are shown in Table 2. The case of Salas was also studied in terms of how the geological composition of the basin influences the physicochemical

characteristics of inland waters (Parra et al. 2012). According to the Spanish Geological and Mining Institute (IGME) the geological stratum of the Salas reservoir comprises biotitic granites and biotytic amphibiotic granodiorites. Biotite is a ferromagnesian phyllosilicate with a high iron content.

2.3.Statistical analysis

The results are mean values ± standard deviation from three independent experiments. Correlations between absorbance and cell concentration were established for each separate culture by polynomial equations as $Y = ax^2 + bx + c$ with the curve-based Pearson product moment correlation coefficient with 5% optimization. Data were analysed by One-way ANOVA using multiple comparisons and the least significant difference. The normality of the data and homogeneity of variances were confirmed before statistical treatments with a Kolmogorov Smirnov test and Levene's test. This was done with the IBM SPSS Statistics 25 program. Differences between years and points and between cultures were analysed using Kruskal–Wallis tests. These are nonparametric statistical tests that assess the differences between three or more independently sampled groups on a single, non-normally distributed continuous variable

3. Results and discussion

3.1.Total algae growth

The correlation between cellular growth and absorbance of the mean of the three samples for each reservoir was positive ($R^2 \geq 95\%$). All the samples were positively related to adjustment with a polynomial equation of degree 2, as shown in Figure 2.

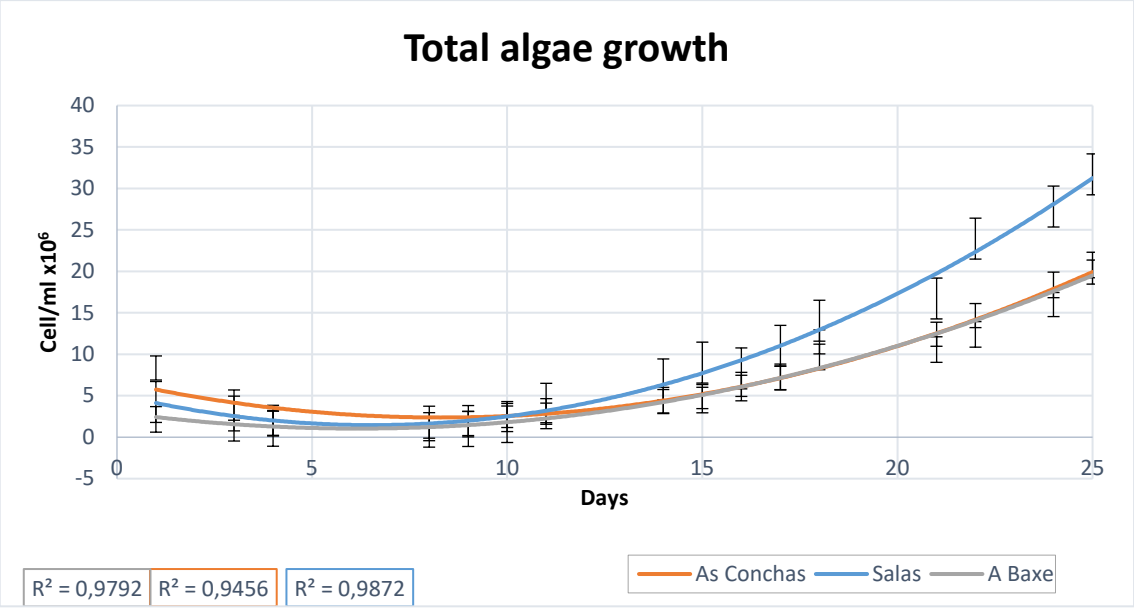


Figure 2: Total growth of algae in the As Conchas, Salas and A Baxe cultures. Each datum is the average of the samples analysed in triplicate and their deviation.

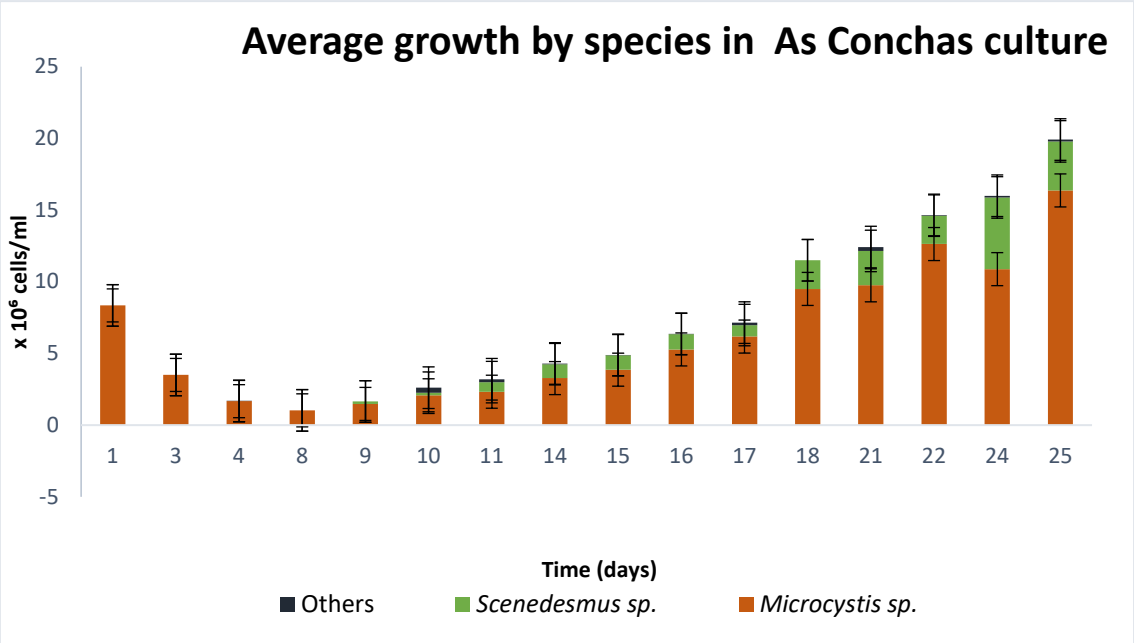
It is noteworthy that the Salas sample with the highest cell growth ($4.25x10^6$ cell/ml the first day of culture to $21.7x10^6$ cell/ml on the last) for the same light and temperature conditions (Table 3). The algae growth of this reservoir was above the average for the other cultures.

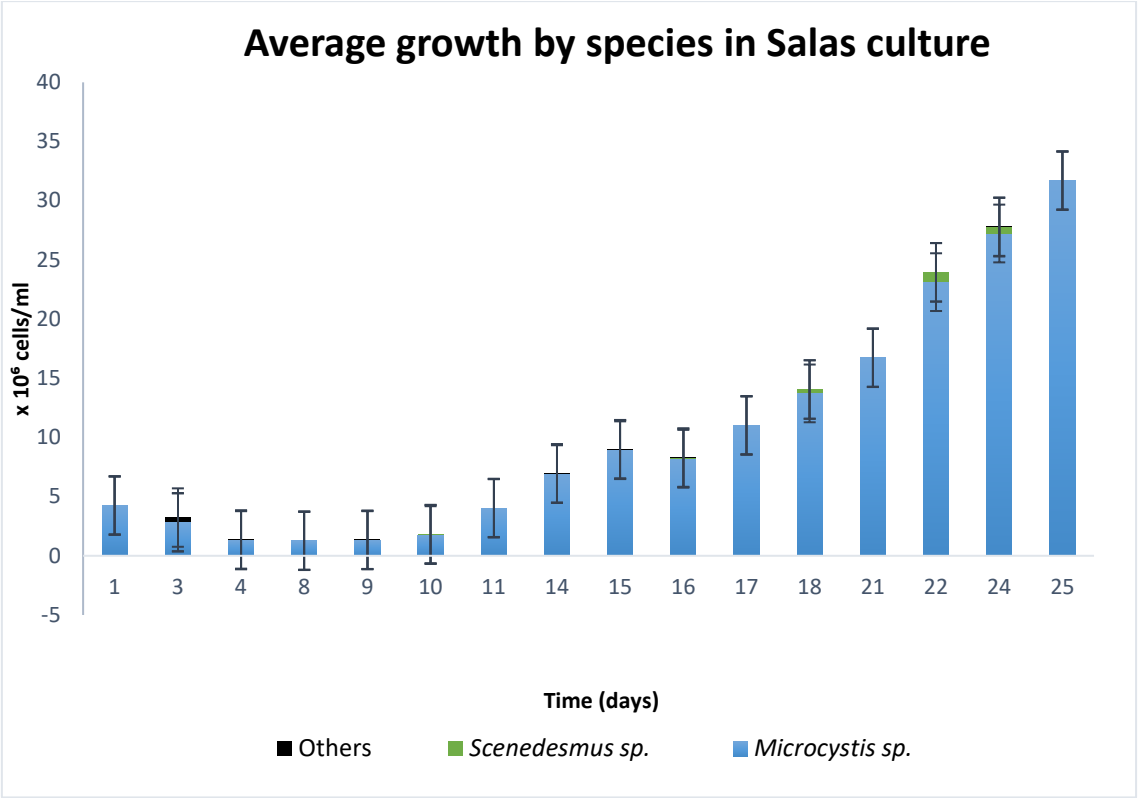
The asymmetry was found to be positive for all three crops at 0.855 for As Conchas, 1.036 for Salas and 1.182 for A Baxe. The Levene's homogeneity test variances show that the variance based on the average of the three cultures is homogeneous with P-value = 0.063 ($P \geq 0.001$). The Kolmogorov-Smirnov normal distribution test shows that the growth curves follow a normal distribution with P-value = 0.195 for As Conchas, P-value = 0.144 for Salas and P-value = 0.200 for A Baxe ($P \geq 0.05$). The ANOVA analysis shows that there are no significant differences in the growth curves of the three crops, which were subject to the same growing conditions although they came from different reservoirs $F_{(2.45)} = 0.872$ with P-value = 0.425 ($P \geq 0.001$).

3.2.Growth of the different species in the algae cultures

Throughout the 25 days of culturing for the three reservoirs studied, it was mainly the cyanobacteria *Microcystis sp.* and the microalgae *Scenedesmus sp.* That developed, as shown in

Figure 3. In the culture for As Conchas other cyanobacteria were also found, such as *Anabaena crassa, Phormidium sp.*, and *Woronichinia naegeliana.* In that for Salas there was *Phormidium sp*. and in that for A Baxe *Aphanizomenon flos aquae*, *Raphidiopsis curvata* and *Coelosphaerium kuetzingianum* were found.





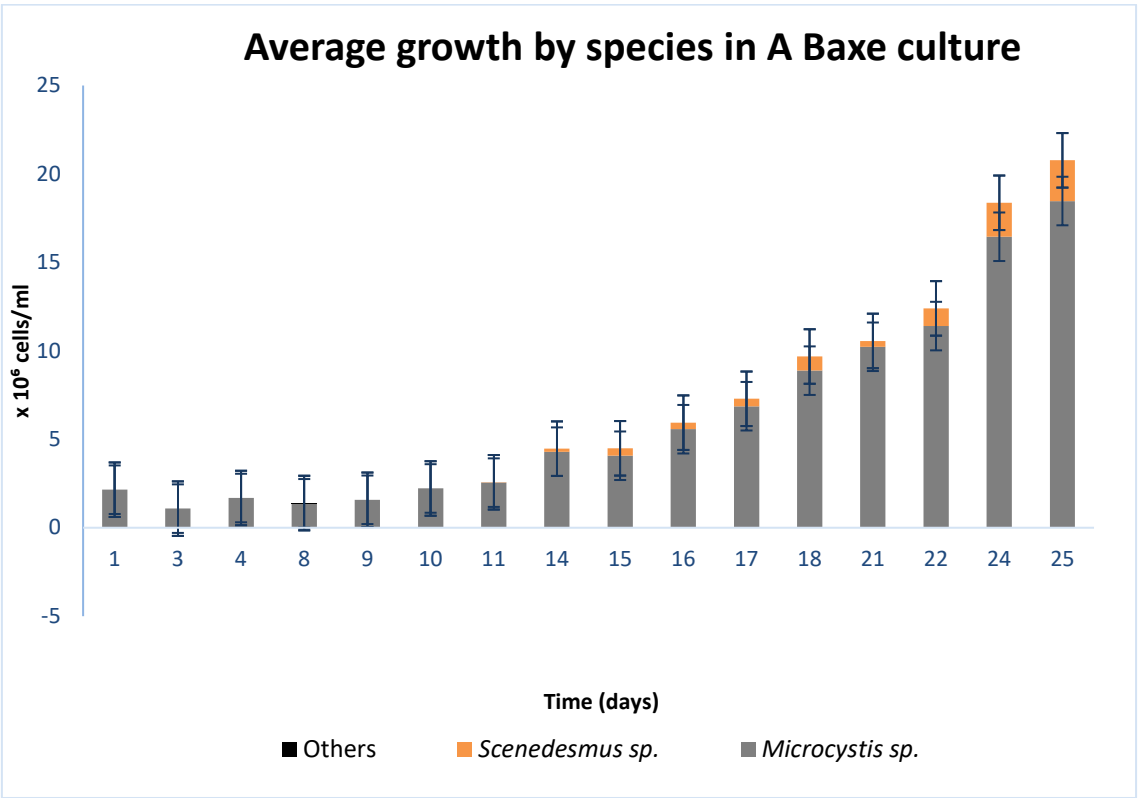


Figure 3: Growth of algae by species in all cultures over the 25 days of culturing. Samples from each reservoir were taken in triplicate. The mean and deviation for each day are shown

At the As Conchas reservoir a high initial concentration of *Microcystis sp.* ($8.35x10^6$ cell/ml) was found, as also detected in other studies (Lago et al. 2016). In the Baxe reservoir, where cyanobacterial proliferations have been detected for more than 10 years, leaving the inhabitants of the Salnés region without drinking water (Álvarez, 2015), the concentration of *Microcystis sp*. was the lowest of the three reservoirs studied ($2.15x10^6$ cell/ml). The first episode of *M. aeruginosa* was detected in 2006 (Cobo, 2008). Of the three water samples from the reservoirs studied, the case of Salas stands out. The water sample from the Salas reservoir had a concentration of *Microcystis sp*. at the beginning of the culture of $4.25x10^6$ cell/ml, and reached the highest concentration after 25 days ($31.70x10^6$ cell/ml). However Lago et al. (2016) did not detect cyanobacteria in this reservoir.

Several factors influence the growth of microalgae in fresh water, reservoirs, rivers and lakes: the most widely studied are temperature and light (Foy et al. 1976). Throughout this study these two factors have remained constant. In the first test, where waters of As Conchas, Salas, and A Baxe were cultured, an average temperature of 21±2°C was maintained for 25 days. Zehnder and Gorham (1960) find the optimum temperature for the growth of *M. aeruginosa* to be approximately 28°C. Robarts and Zohary (1987) find that the growth of the *Microcystis sp.* cyanobacterium is severely limited at temperatures below 15° C and is optimal at temperatures around 25°C. Other studies highlight that such cultures do not survive in temperatures above 29°C (Reynolds (2006); Paerl and Paul (2012)). Therefore, the temperature for growth can be limited for *M. aeruginosa* between 15-28°C, which means that we are within the optimum temperature range. Another microalga frequently found was the genus *Scenedesmus sp*., for which the optimum temperature is between 10 and 30°C (Xin et al. 2011). Yang et al. (2018) conduct a study on the competition between *Microcystis aeruginosa* and *Scenedesmus obliquos,* and reflects that in a temperature range between 15-35ºC, *Scenedesmus obliquos* at 15 ºC competes better. In the 20-30ºC range both compete, with *Scenedesmus sp* being dominant in the first phase of the crop but later being replaced by *Microcystis aeruginosa* when the temperature increases. In our

crops, note that although *Scenedesmus* sp. increases the cyanobacterium Microystis sp. remains dominant throughout the 25 days (Figure 3). This dominance is very important for the ecosystem and for the possible treatment of water, since it is the dominance of a toxic species over a non-toxic one.

Our experimental results show that pH increases as the cell culture grows in the samples from all three reservoirs studied, with a strong positive correlation with R^2=0.9907 for As Conchas, 0.9566. for Salas and 0.9612 for A Baxe. The samples of water cultured in the laboratory arrived with an initial pH value of 7.94 from the As Conchas reservoir. This figure increased to 9.24 on the last day of culturing. The samples of water isolated in the laboratory arrived with an initial pH value of 5.72 from the Salas reservoir, increasing to 9.02. For those from the A Baxe reservoir the initial pH was 7.95, increasing to 9.07.

The As Conchas, Salas and A Baxe cultures all increased their pH based on their cell growth. Salas showed most variation in its cellular concentration and had the lowest pH. Increases in pH depending on increases in cell density may be due to the fact that at high productivity rates the consumption by the phytoplankton of CO_2 dissolved in water increases. This detraction leads to the formation of HCO_3 due to the buffering capacity of water, which increases its concentration against CO_2. The assimilation of HCO^{3-} releases OH^- ions, increasing pH (Martinez et al. 2000). The absorption as a nutrient of HCO^{3-} does not occur in all cases: it depends on whether it passes through the membrane and the carbonic anhydrase enzyme acts in the cytoplasm (Moretó, 1997). This makes certain species more competitive in that they are able to use this as an alternative source of inorganic carbon (Carvajal Cruz, 2011). This increase is counteracted by the oxidation of organic matter by zooplankton as predates phytoplankton, consumes O_2 and introduces CO_2 into the medium (Margalef, 1995), causing pH increases at high rates of productivity to be lower.

Studies on the effect of pH on the growth of *Microcystis aeruginosa* reveal that acidic environments inhibit it, and when the environment becomes neutral or alkaline it leads to rapid growth (Wang et al. 2011), even allowing the dominance of cyanobacteria over microalgae. Other studies reveal that *Scenedesmus sp*. usually develops in ranges of pH 6-9, with 9 being the highest

concentration for isolated strains. However, in competition with *Microcystis aeruginosa* and at similar initial concentrations, when the pH reaches between 7 and 9, *Scenedesmus sp*. inhibits its growth and *Microcystis sp.* Is a better competitor (Yang et al. 2018). In all three cultures here the pH range moved in the range of 6-9. As indicated by Yang et al. (2018), the cyanobacterium *Microcystis sp.* dominates the microalga *Scenedesmus sp.*.

3.3.Total algae growth with sediments

In all five samples the correlation is positive (R^2> 0.90). This correlation is shown in a polynomial equation $y = ax^2 + bx + c$ in Table 4 below. With these data we were able to estimate the cell concentration from an absorbance reading in each culture.

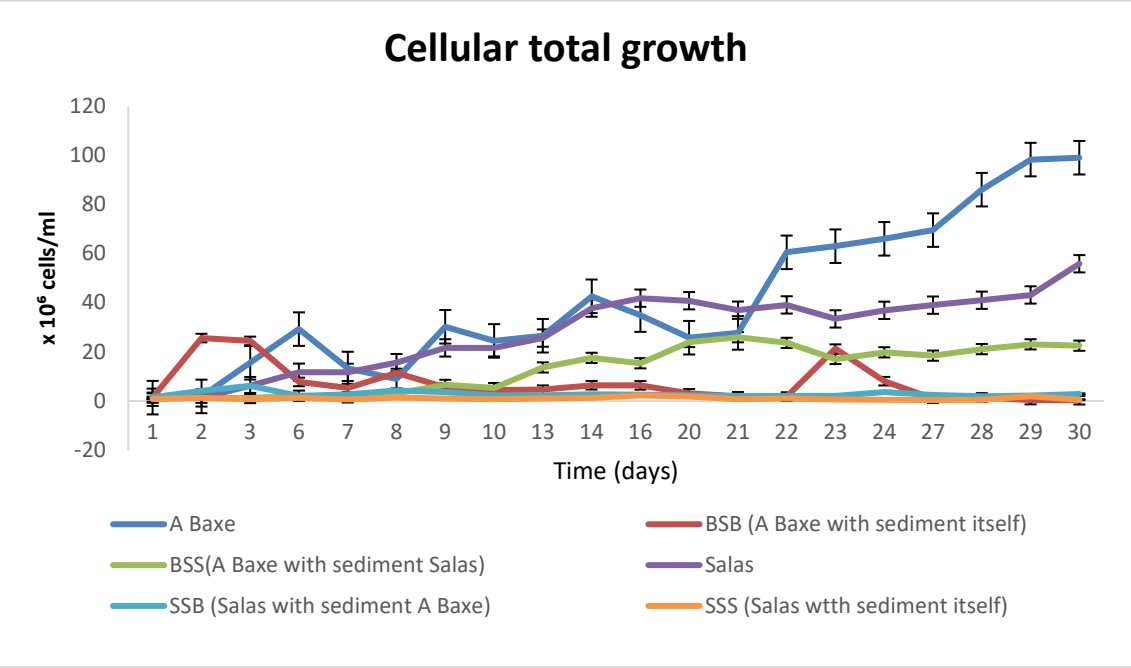


Figure 4: Growth of algae for different crops with sediments. Each sample was analyzed in triplicate for 30 days of data collection.

Once the culture medium was added the SSS (Salas with sediment from Salas) crop showed low concentrations throughout the study. However, when the sediment from the reservoir was varied or removed it reached high concentrations like those of Salas and SSB (Salas with sediment from A Baxe reservoir), as can be seen in Figure 4. Salas showed higher concentrations ($55.85x10^6$

cell/ml) than SSB ($2.85x10^6$ cells/ml) (see table 5). The BSS (Baxe with sediment from Salas reservoir) culture stood out throughout the study in that the percentage of actinobacteria increased over that of *Microcystis sp*. On the first day of culturing all the microalgae counted belonged to the genus *Microcystis sp*. while for the last day 60% were actinobacteria and almost 40% *Microcystis sp*. (Figure 5). A Baxe obtained the highest values of cell growth, while BSB remained constant. It is an important result, considering that the concentration of *Microcystis sp*. decreased. Therefore, it would be advisable to collect samples during periods of blooms in reservoirs, to verify that this same relationship happens in the study areas.

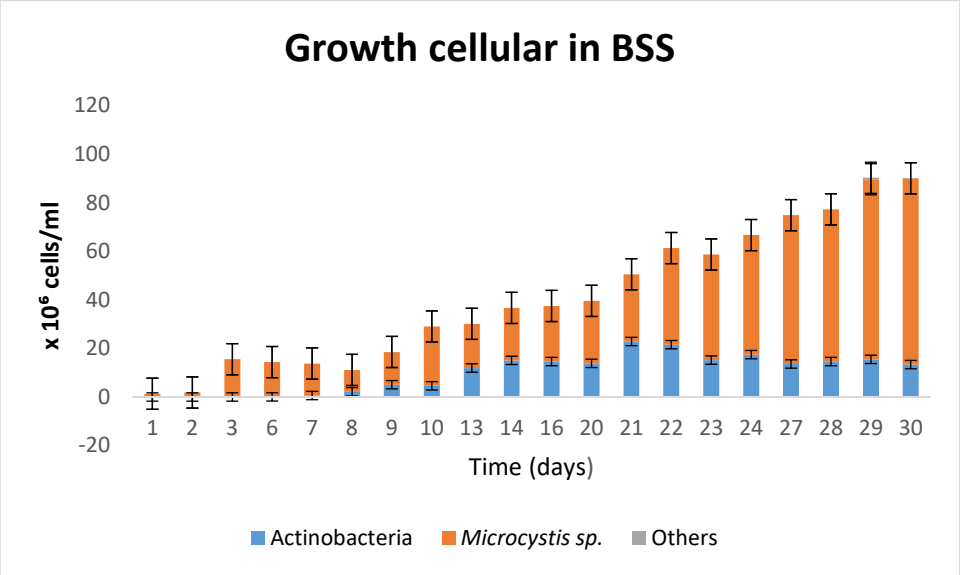


Figure 5: This graph shows cell growth in the case of BSS over the 30 days of culturing.

The homogeneity of the cultures was assessed with a statistical analysis of homogeneity of Levene variances and Kolmogorov-Smirnov normality. The cultures from A Baxe and Salas both showed positive results, with P = 0.019 for Levene and P = 0.200 for the K-S test. An ANOVA analysis was performed and showed homogeneity between the two crops with $F_{(1.38)} = 1.714$ and P = 0.298. Waters with different initial characteristics developed crops with homogeneous growth.

For SSB and BSB the results were positive in terms of homogeneity and negative in terms of normality with P = 0.390 for Levene and P = 0.000 for the K-S test. A Kruskal-Wallis analysis conducted for independent samples showed homogeneity in terms of the average of the two crops

with Hks = 0.681 and P = 0.409 and in terms of the median P = 1,000. Waters with different initial characteristics developed cultures with homogeneous growth rates, apparently, when they were conditioned by the same parameters in the laboratory culture.

SSS and BSS crops gave negative results in terms of homogeneity and normality with P = 0.000 for Levene and P = 0.000 for the K-S test. A Kruskal-Wallis analysis performed for independent samples showed no homogeneity in terms of the average of the two crops, with Hks = 26.156 and P = 0.000, or in terms of the median, with P = 0.000. Therefore, there are significant differences between the two in terms of growth.

We found that when a disintegration as per Janse et al. (2004) was performed there was a lower concentration (e.g. A Baxe, $20.77x10^6$ cells/ml) and greater absorbance (1.429) (Table 3) than if no disintegration was performed ($99.3x10^6$ cells/ml, and absorbance of 0.704) (Table 6). This may be because in the first case the absorbance is mediated before disintegration. When a disintegration is carried out before the absorbance measurement, the cells are separated and cease to be colonies, so they are more diluted in the water and the absorbance is lower.

There are several studies that deal with the chemical precipitation of phosphates with iron according to the pH of the medium (acid) and its oxidising capacity (Acevedo-Sandoval et al. 2004), in which insoluble salts precipitate (Martinez et al. 2001), forming a brown layer in the middle bed. This may be why the concentration of phosphates in the Salas reservoir was low, at 0.26 mg/l. Phosphate is one of the most essential nutrients for phytoplankton growth and a continued deficiency of this compound can lead to low photosynthetic efficiency (Crettaz, 2018) and even growth inhibition (limiting factor). On the other hand, according to the IGME, the strata at the A Baxe reservoir comprise biotitic granites and biotitic granodiorites on its southwest slope and quartzites, small hands and ampelites on its northeast slope. This could explain why the cultures with A Baxe sediment did not produce high cell growth. With cultures made up of small samples (only 600 ml) it is easier for this effect to occur.

3.4.Growth of the different species in the algae cultures with sediments

For the A Baxe reservoir, almost 97.97% of the growth belonged to the genus *Microcystis sp*. For the Salas reservoir, the genus that accounted for most of the cell count was *Microcystis sp*. (99.36% on the last day).

Growth in the BSB culture (A Baxe with sediment from the same location) began very irregularly, with most of the cultures being more constant. Much of the algae that developed in this crop were too large to be counted in the Neubauer chamber and had to be observed under the microscope with the help of a slide and a coverslip. Most of the cells counted belonged to the genus *Microcystis sp*. Growth in the SSB reservoir culture remained stable throughout the culturing as regards *Microcystis*. In the BSS culture, *Microcystis sp.* Accounted for 40.17% and actinobacterias for 59.83%. *Microcystis sp*. (71.79%), and actinobacterias (28.20%) developed in the SSS culture.

pH was studied as the culture progressed (Figure 6). For the culture from A Baxe the initial pH was 6.84 and the highest reading was 8.53. For BSB it reached 9.56 and for BSS (A Baxe with sediment from Salas) it reached 8.1. For the culture from Salas the initial pH was 6.56, rising to 8.89. The SSB culture reached 10.09 and the SSS culture (Salas with sediment from the same location) reached 7.65.

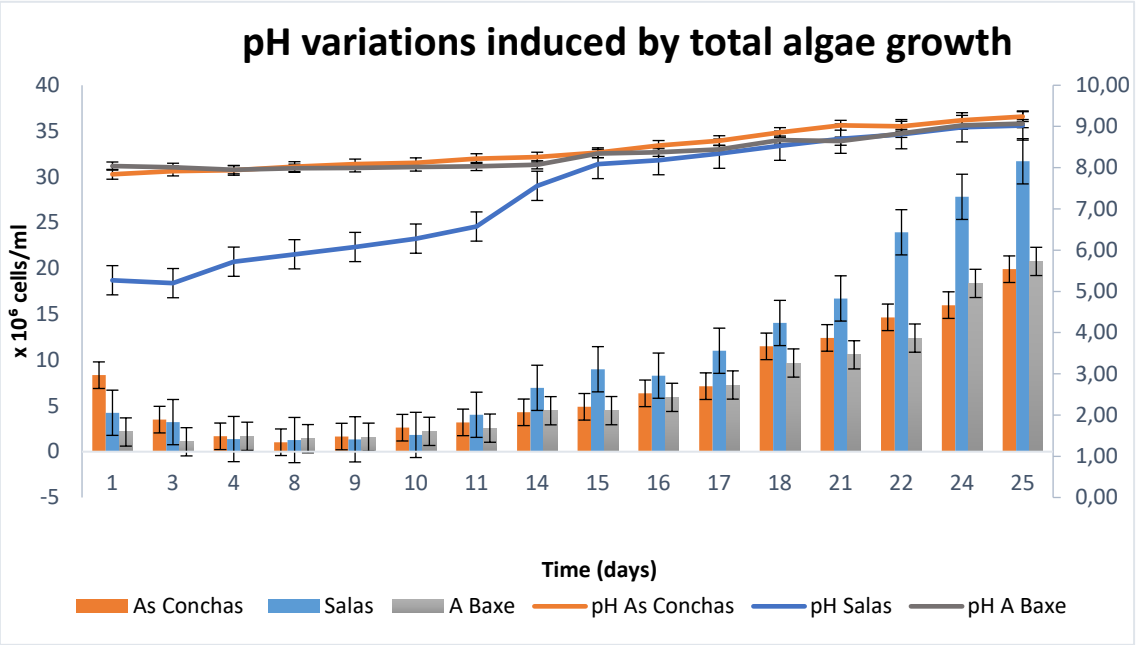


Figure 6: Total growth for each crop. As Conchas, Salas and A Baxe are represented together with the pH variation.

Throughout the cultures from Salas, SSS and BSS it was observed that as the crop progressed, actinobacteria developed, specifically the genus *Streptomyces*, and that the growth of *Microcystis sp*. was inhibited. This genus is very common both on land and in water and can appear in sediment or along riverbanks associated with vegetation, cyanobacteria themselves or green algae. (González Iglesias, 2013). In the Salas reservoir, barley straw is used to treat runoff after forest fires in the basin. The addition of barley straw to soil results in an increase in the counts of actinobacteria and fungi (Baćmaga et al. 2013). There could be a direct link to the inhibition of cyanobacteria when using barley straw where they are part of a wide range of microorganisms that produce antimicrobial substances. *Streptomyces exfoliatus* causes 50% of *Anabaena sp.*, *Microcystis sp.* and *Oscillatoria sp.* mortality (Sigee et al. 1999). Different studies highlight that barley straw can be used in the control of blooms (Newman et al. 1993). If anticianobacterial activity is due to oxidised phenolic compounds (Solano, 2018), then the effect may also be produced by the breakdown of lignin (source of phenolic and tannin compounds) from barley straw (Ball et al. 2001). Zhang et al. (2016) report that the genus *Streptomyces* can secrete tryptamine and tryptoline. These substances showed similar or higher (up to 2.5-fold) algicidal activities on most of the bloom-forming cyanobacteria strains tested than a commonly used algicide $CuSO_4$ ($P<0.05$, or $P < 0.01$), suggesting their potential as alternative reagents for treating CyanoHABs. Moreover, Zhang et al. (2016) conclude that tryptamine and tryptoline treatments significantly alter the internal and external contents of microcystin-LR. In the present paper, the concentration of microcystin-LR is not studied, but this is an interesting issue for future studies.

The As Conchas reservoir has a high initial value of cyanobacterias compared to the Salas reservoir, but the latter has the advantage of having actinobacteria present in the sediment. The Salas-Conchas reversible power plant project (approved on May 5, 2017 (BOE, 2017)) could thus compromise the ecological status of Salas water.

4. CONCLUSIONS

For the same culture conditions, samples from the As Conchas, Salas and A Baxe reservoirs showed homogeneity in their cell growth curves, with high densities at 16 days. Water from the Salas reservoir had the lowest initial concentration but reached the highest concentration at the end of the culturing. Our experimental results show increases in pH as the cell culture grows in the samples, with a strong positive correlation. Once they reached their maximum cell density all cultures had pH levels close to 9, even Salas, which started with a pH of 5.27. In non-limiting environments and the same culture conditions, waters from the same reservoir grown with two different sediments showed significant differences in their cell growth. The sediment from the basin in which a reservoir is located plays an important role in its characteristics and biogeochemical cycles, and consequently in the development of phytoplankton. In all the cultures in which sediment was used, microalgal growth was inhibited. The growth of actinobacteria in the cultures with sediment from Salas stood out, where *Microcystis sp.* was inhibited. The cultures with A Baxe sediment showed the highest pH and the lowest concentration. Acidic pH levels and ferromagnesian sediments from biotites can lead to phosphate being a limiting factor for phytoplankton, due to the formation and sedimentation of insoluble salts of ferric phosphate.

ACKNOWLEDGEMENTS

We thank professors Carlos Souto and María Eugenia López (University of Vigo) for providing necessary material, and as Francisco Abilleira for aid with the necessary infrastructure.

Figure 1: Geographic location of As Conchas, Salas and A Baxe basins. Drainage network and land uses are also shown.

Figure 2: Total growth of algae in the As Conchas, Salas and A Baxe cultures. Each datum is the average of the samples analysed in triplicate and their deviation.

Figure 3: Growth of algae by species in all cultures over the 25 days of culturing. Samples from each reservoir were taken in triplicate. The mean and deviation for each day are shown.

Figure 4: Growth of algae for different crops with sediments. Each sample was analyzed in triplicate for 30 days of data collection.

Figure 5: This graph shows cell growth in the case of BSS over the 30 days of culturing.

Figure 6: Total growth for each crop. As Conchas, Salas and A Baxe are represented together with the pH variation.

Table 1: Information on the reservoirs studied: As Conchas, Salas and A Baxe.

Table 2: Composition of the sediments from the Salas and A Baxe reservoirs. The composition of the ash collected at the Salas reservoir is also included.

Table 3: a) Information on the cultures **b)** Parameter equations of the crops studied

Table 4: a) Parametric equations of the cultures with sediment obtained from the cellular representation of the average for each crop over the 30 days of cultivation. **b)** Information on the sediment cultures.

Table 1: Information on the reservoirs studied: As Conchas, Salas and A Baxe.

RESERVOIR	RIVER	UTM	CHARACTERISTICS	Land uses	Average rainfall (mm)	Average temperatures	Watershed altitudes range (m)
As Conchas	Limia	82385 E 4655927 N	Gravity dam; watershed area 978 km²; capacity 78.33 hm³; hydroelectic, thermal baths and fishing uses.	30.7% moors and heathland 19.1% non-irrigated arable land 15.2% broad-leaved forest 10.7% agriculture or natural vegetation 24% other land uses	1200	5.9°C in January 19.3 °C in July-August	537-1533
Salas	Salas (Limia efluent)	89525 E 4653497 N	Buttresses dam; watershed area 145 km²; capacity 74.86 hm³; hydroelectric and fishing uses.	49.4% moors and heathland 14.9% agriculture or natural vegetation 12.0% broad-leaved forest 24% others land uses	1100	4.4°C in January 18.0°C in July-August	142-809
A Baxe	Umia	533823 E 4716735 N	Gravity dam; watershed area 182 km²; capacity 70.00 hm³; hydroelectic, flow regulation and fishing uses.	35% Broad-leaved forest, 24.8% Complex cultivation patterns 15.6% Moors and heathland 10% Coniferous forest 15% Other land uses	1400	7 °C in January 20.5 °C in July-August	103-802

8

9 **Table 2:** Composition of the sediments from the Salas and A Baxe reservoirs. The composition of the ash collected at the Salas reservoir is also included.

10

Component	Salas sediment	Salas ash soil	A Baxe sediment
%N	<0.05	1.16	0.05
%C	0.11	17.76	0.74
g/kg Na	0.17	0.29	0.2
g/kg K	1.2	2.7	5.7
g/kg Fe	16.3	27.9	30.1

Table 3: a) Information on the cultures **b)** Parameter equations of the crops studied

a)	As Conchas	Salas	A Baxe
Time (days)	25	25	25
Temperature (± 2)	21	21	21
Cell accounting (cell/µl) x10^6 first day	8.35	4.25	2.15
Cell accounting (cell/µl) x10^6last day	19.92	21.7	20.77
Absorbance first day (nm)	0.088	0.066	0.057
Absorbance last day (nm)	1.917	2.218	1.429

b)	**Equation Parameters**			
Culture	**a**	**b**	**c**	**R^2**
As Conchas	0.398	-0.2681	0.8982	0.9796
Salas	0.0751	-0.7165	1.7443	0.9882
A Baxe	0.0448	-0.4236	1.7924	0.9755

Table 4: a) Parametric equations of the cultures with sediment obtained from the cellular representation of the average for each crop over the 30 days of cultivation. **b)** Information on the sediment cultures.

a)

		Equation Parameters			
Culture	**Code**	**a**	**b**	**c**	**R^2**
A Baxe	Baxe	0.00005	0.0048	0.0454	0.966
A Baxe with sediment A Baxe	BSB	0.000009	0.0003	0.0654	0.914
A Baxe with sediment Salas	BSS	-0.0029	0.0361	0.0332	0.008
Salas	Salas	0.0001	0.0003	0.0796	0.947
Salas with sediment A Baxe	SSB	0.0015	-0.001	0.0604	0.921
Salas with sediment Salas	SSS	0.0306	-0.0483	0.0732	0.907

b)

	Baxe	BSB	BSS	Salas	SSB	SSS
Time (days)	30	30	30	30	30	30
Temperatura (± 3)	19	19	19	19	19	19
Cell accounting (cell/µl) $x10^6$ first day	1.35	1.35	1.35	0.82	0.82	0.82
Cell accounting (cell/µl) $x10^6$ last day	99.3	0.3	22.5	55.85	2.85	0.55
Absorbance first day (nm)	0.089	0.089	0.089	0.063	0.063	0.063
Absorbance last day (nm)	0.704	0.062	0.136	0.401	0.073	0.064

REFERENCES

Acevedo-Sandoval, O., Ortiz-Hernández, E., Cruz-Sánchez, M., & Cruz-Chávez, E. (2004). El papel de óxidos de hierro en suelos. Terra Latinoamericana, 22(4), 485-497

Álvarez, X. (2015). Modelo conceptual de la eutrofización y proliferación de cianobacterias. Un caso de estudio en el embalse de A Baxe (Doctoral dissertation, Universidade de Vigo).

Álvarez, X., Valero, E., Santos, R. M., Varandas, S. G. P., Fernandes, L. S., & Pacheco, F. A. L. (2017). Anthropogenic nutrients and eutrophication in multiple land use watersheds: Best management practices and policies for the protection of water resources. *Land Use Policy*, *69*, 1-11.

Avery, S. V. (1996). Fate of caesium in the environment: distribution between the abiotic and biotic components of aquatic and terrestrial ecosystems. Journal of Environmental Radioactivity, 30(2), 139-171.

Baćmaga, M., Kucharski, J., Wyszkowska, J., & Tomkiel, M. (2013). Biological properties of soil contaminated with the Aurora 40 WG herbicide. Publisher UWM, 163.

Ball, A. S., Williams, M., Vincent, D., & Robinson, J. (2001). Algal growth control by a barley straw extract. Bioresource Technology, 77(2), 177-181.

Bell, S. G., & Codd, G. A. (1994). Cyanobacterial toxins and human health. Reviews in Medical Microbiology, 5(4), 256-264.

Beltrán-Rocha, J. C., Guajardo-Barbosa, C., Barceló-Quintal, I. D., López-Chuken, U. J., Nava-Olvera, R., Mateo-Cid, L. E., .&Monreal-Gómez, M. A. (2017). Biotratamiento de efluentes secundarios municipales utilizando microalgas: Efecto del pH, nutrientes (C, N y P) y enriquecimiento con CO 2. Rev. biol. mar. oceanogr, 52(3), 417-427.

Carpenter, S. R., Stanley, E. H., &Vander Zanden, M. J. (2011). State of the world's freshwater ecosystems: physical, chemical, and biological changes. Annual review of Environment and Resources, 36, 75-99.

Carvajal Cruz, G. E. (2011). Ocurrencia de floraciones de cianobacterias tóxicas en cuerpos de aguadulce, Argentina (Doctoral dissertation, Facultad de Ciencias Exactas).

Chislock, M. F., Doster, E., Zitomer, R. A., & Wilson, A. E. (2013). Eutrophication: causes, consequences, and controls in aquatic ecosystems. Nature Education Knowledge, 4(4), 10.

Cobo, F. 2008. Floracións de Cianobacterias tóxicas en augas continentais. CERNA 54: 24-28

Codd, G. A. (2000). Cyanobacterial toxins, the perception of water quality, and the prioritisation of eutrophication control. Ecological engineering, 16(1), 51-60.

Crettaz Minaglia, M. C. (2018). Estudio del crecimiento de Microcystis aeruginosa y de la producción de Microcystina en cultivo de laboratorio (Doctoral dissertation, Facultad de Ciencias Exactas).

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., ... & Thornbrugh, D. J. (2008). Eutrophication of US freshwaters: analysis of potential economic damages.

Duque Escobar, G. (2012). Manual de geología para ingenieros

Filley, T. R., Nierop, K. G., & Wang, Y. (2006). The contribution of polyhydroxyl aromatic compounds to tetramethylammonium hydroxide lignin-based proxies. Organic Geochemistry, 37(6), 711-727.

Foy, R. H., Gibson, C. E., & Smith, R. V. (1976). The influence of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. *British phycological journal*, *11*(2), 151-163.

92 Gallego, A., Hospido, A., Moreira, M. T., & Feijoo, G. (2009). Quantification of eutrophic
93 aerial compounds in Galicia (NW Spain): Part 2-NOx inventory. Atmósfera, 22(2), 161-174.

94 Giné, D. S. (2012). Ramsar wetlands in Spain: reflections on the occasion of their 30th
95 anniversary. Investigaciones Geográficas, (57), 129.

96 González Iglesias, V. (2013). Producción de antibióticos en Streptomyces asociados a
97 organismos marinos.

98 Guillard, R. R. L., 1975. Culture of phytoplankton for feeding marine invertebrates. In W. L.
99 Smith & M. H. Chantey (eds), Culture of Marine Invertebrate Animals. Plenum Publishers,
100 New York: 29–60

101 Harada, K. I. (2004). Production of secondary metabolites by freshwater
102 cyanobacteria. Chemical and Pharmaceutical bulletin, 52(8), 889-899.

103 J. McKight, P. E., & Najab, Kruskal-wallis test., Corsini Encycl. Psychol. 1–1 (2010).

104 Janse, I., Kardinaal, W. E. A., Meima, M., Fastner, J., Visser, P. M., &Zwart, G. (2004). Toxic
105 and nontoxic Microcystis colonies in natural populations can be differentiated on the basis of
106 rRNA gene internal transcribed spacer diversity. Applied and Environmental
107 Microbiology, 70(7), 3979-3987.

108 Lago, L., Barca, S., Vieira-Lanero, R., &Cobo, F. (2016). Floraciones de cianobacterias y
109 valores de microcistina-LR sestónica y disuelta en embalses de la cuenca hidrográfica del
110 Miño-Sil (NW-España). ÍNDICE–INDEX.

111 Li K, Song L, Wan N (2003) Studies on recruitment and growth characteristic of Microcystis
112 in sediment. Acta Hydrobiologica Sinica/ Chung-kuoko Hsueh Yuan Shui Sheng Shengwu
113 yen Chiu so 28(2):113–118

114 Luna, L. G., &Carmenate, Z. R. (2009). Microalgas como biomonitores de contaminación.
115 Universidad de Oriente (Cuba).

116 Marbello Pérez, R. V., Toro Botero, F. M., & Aguirre Ramírez, N. J.2012 Modelo de calidad
117 del agua en ecosistemas lénticos considerando equilibrio químico entre especies
118 constituyentes.

119 Margalef, R. 1995. Ecología. Ediciones Omega, SA. Barcelona. 951 p.

120 Martinez ME, S Sanchez, JM Jimenez, F El Yousfi& L Munoz. 2000. Nitrogen and
121 phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus.
122 Bioresource Technology 73: 263-272.

123 Moretó, M. (1997). Fisiologia cellular: ensenyament de farmàcia (Vol. 72). Edicions
124 Universitat Barcelona

125 Newman, J. R., & Barrett, P. R. F. (1993). Control of Microcystis aeruginosa by decomposing
126 barley straw. Journal of aquatic plant Management, 31, 203-203.

127 Paerl HW, Paul VJ (2012) Climate change: links to global expansion of harmful
128 cyanobacteria. Water Res 46(5):1349–1363

129 Paerl, H. W., Hall, N. S., & Calandrino, E. S. (2011). Controlling harmful cyanobacterial
130 blooms in a world experiencing anthropogenic and climatic-induced change. Science of the
131 Total Environment, 409(10), 1739-1745.

132 Palmer, M. A., Covich, A. P., Lake, S. A. M., Biro, P., Brooks, J. J., Cole, J., ... & Verhoeven,
133 J. O. S. (2000). Linkages between Aquatic Sediment Biota and Life Above Sediments as
134 Potential Drivers of Biodiversity and Ecological Processes: A disruption or intensification of
135 the direct and indirect chemical, physical, or biological interactions between aquatic sediment
136 biota and biota living above the sediments may accelerate biodiversity loss and contribute to
137 the degradation of aquatic and riparian habitats. AIBS Bulletin, 50(12), 1062-1075.

138 Parra, R. R., Roeser, H. M. P., Leite, M. G. P., NaliniJr, H. A., Guimarães, A. T. A., Pereira,
139 J. C., & Friese, K. (2012). Influência antrópica na geoquímica de água e sedimentos do Rio
140 Conceição, Quadrilátero Ferrífero, Minas Gerais-Brasil. Geochimica Brasiliensis, 21(1).

141 Pérez, G. R., & Restrepo, J. J. R. (2008). Fundamentos de limnología neotropical (Vol. 15).
142 Universidad de Antioquia.

143 Reynolds, C. S. (2006). The ecology of phytoplankton. Cambridge University Press.

144 Robarts, R. D., & Zohary, T. (1987). Temperature effects on photosynthetic capacity,
145 respiration, and growth rates of bloom-forming cyanobacteria. New Zealand Journal of
146 Marine and Freshwater Research, 21(3), 391-399.

147 Rodrigues, Ú. G. B. D. S. (2017). Macrófitas aquáticas na área de transição entre o rio Areias
148 e o reservatório da Usina Hidrelétrica Luís Eduardo Magalhães-Tocantins: cobertura,
149 biomassa e macroinvertebrados asociados.

150 Rosso, L., &Giannuzzi, L. (2011). Factores ambientales y antropogénicos que afectan la
151 formación de floraciones de cianobacterias y cianotoxinas. biental.

152 Schindler, D. W. Eutrophication and recovery in experimental lakes: implications for lake
153 management. Science **174**, 897-899 (1974).

154 Schmid, A. G. (2008). Diferenciación espacial en la metodología de Análisis del Ciclo de
155 Vida: desarrollo de factores regionales para eutrofización acuática y terrestre. Univ Santiago
156 de Compostela.

157 Schmid, A. G., Quintana, A. H., Moreira, M. T., & Costa, G. F. (2009). Quantification of
158 eutrophic aerial compounds in Galicia (NW Spain). Part 1-NH, inventory. Atmósfera, 22(2),
159 141-160.

160 Sigee, D. C., Glenn, R., Andrews, M. J., Bellinger, E. G., Butler, R. D., Epton, H. A. S., &
161 Hendry, R. D. (1999). Biological control of cyanobacteria: principles and possibilities. In The
162 Ecological Bases for Lake and Reservoir Management (pp. 161-172). Springer, Dordrecht.

163 Solano Jiménez, R. (2018). Determinación del efecto alelopático del extracto de Oryzasativa
164 L. sobre el crecimiento de cianobacterias productoras de toxinas (Master'sthesis).

UNE-EN 15204:2007. Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)

UNE-EN 16698:2016. Water quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters

Wang X, Hao CB, Zhang F, Feng CP, Yang YN (2011) Inhibition of the growth of two blue-green algae species (Microsystis aruginosa and Anabaena spiroides) by acidification treatments using carbon dioxide. Bioresour Technol 102(10):5742–5748

Xin L, Hong-Ying H, Yu-Ping Z (2011) Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. Bioresource Technol 102(3):3098–3102

Yang, J., Tang, H., Zhang, X., Zhu, X., Huang, Y., & Yang, Z. (2018). High temperature and pH favor Microcystis aeruginosa to outcompete Scenedesmus obliquus. Environmental Science and Pollution Research, 25(5), 4794-4802.

Yang, Y., & Gao, K. (2003). Effects of CO2 concentrations on the freshwater microalgae, Chlamydomonas reinhardtii, Chlorella pyrenoidosa and Scenedesmus obliquus (Chlorophyta). Journal of Applied Phycology, 15(5), 379-389.

Zehnder A, Gorham PR (1960) Factors influencing the growth of Microcystis aeruginosa Kütz. Emend. Elenkin. Can J Microbiol 6(6):645–660.

Zhang, B. H., Ding, Z. G., Li, H. Q., Mou, X. Z., Zhang, Y. Q., Yang, J. Y., ... & Li, W. J. (2016). Algicidal activity of metabolites from Streptomyces eurocidicus JXJ-0089 and their effects in the physiology of Microcystis. Applied and Environmental Microbiology.